

Reagent or Bead Rails feed both processors. Rail access to the processors can be controlled by valves Vr and Vb. During reagent processing (enzyme reactions), Vr opens and Vb may be closed. During bead-based clean-up, the reverse applies, that is, Vr may be closed and Vb may be open. Each rail can access four different input wells and one waste well, via valves Vr1-4, and VrW, respectively. Each processor can have a sample input well (Sample), two output intermediate processing wells (Out1, Out2), and two eluate output wells E11 and E12. Processors can also have two pumps (Pump, BPump), both of which can actuate fluid transfer. Pump can be used for routine pumping operations while BPump can be used mainly as a bead collection reservoir. The fabrication parameters for the microfluidic chip can be 75 μ m channel depth, 250 μ m (final) fluid channel width. As described below, the pneumatic layer of BPump can be milled-out to a depth of 500 μ m. Pump and BPump pump stroke volumes can be approximately 0.5 μ l and 1 μ l, respectively.

[0092] In some embodiments, the chip functions in conjunction with pneumatic and fluidic manifolds. The pneumatic manifold can mate with pneumatic wells on the bottom surface of the chip, connecting them to either vacuum or positive pressure sources through computer-controlled solenoid valves. The pneumatic manifold can also position magnets underneath BPumps. The fluidic manifold can mate input/output ports to the fluidic wells on the top surface of the chip. Wells Out1 and Out2, however can be used for intermediate processing, and these can connect instead to reaction mixing/incubation reservoirs in the fluidic manifold.

[0093] The valves and pumps can be used to move materials within the components described herein, including a fluidic manifold, a microfluidic chips, and a pneumatic manifold. FIG. 20 illustrates how a reaction comprising Reagent 1 and Sample may be assembled in Out1 by 4-cycle pumping. Assume all valves may be initially closed. In Cycle A, valves Vr1 and Vr can open, allowing Pump to draw Reagent 1 from well Ras1R with a down-stroke (vacuum applied to Pump). Reagent in Ras1R can be drawn into Pump. In Cycle B, valves Vr1 and Vr can be closed and valve V2 can be open, allowing Pump to expel its contents into the Out1 reservoir with an up-stroke (positive pressure applied to Pump). Reagent in Pump can be expelled into Out1 reservoir. In Cycle C, RNA in Sample can be drawn into Pump. In Cycle D, RNA can be expelled into Out1 reservoir. Cycles C and D, operate analogously; the only difference is that Pump is filled from Sample in cycle C. Cycles A, B, C, D are repeated until a sufficient volume has been pushed into Out1. Note that the Reagent 1-to-Sample mixing ratio can be determined by the ratio of cycles AB:CD. In the process described above, the mixing ratio is 1:1, but it can in principle be any integral ratio. Finally, similar procedures can be used to mix any of the reagents (Ras1-4) with Sample, by substituting the appropriate valve for Vr1. Mixing can be promoted by the generation of multiple component interfaces, and by turbulence associated with pumping and fluid flow in chip wells. Mixing can occur due convection and diffusion at multiple interfaces due to sequential layering of reagent and RNA in Out1 reservoir.

C. Pneumatic Manifolds

[0094] A pneumatic manifold can be used to mate the pneumatic lines of a microfluidic chip to external pressure sources. The pneumatic manifold can have ports that align with ports on the pneumatics layer of the microfluidic chip and ports that can be connected to tubing that connect to the external pres-

sure sources. The ports can be connected by one or more channels that allow for fluid communication of a liquid or gas, or other material between the ports.

[0095] The pneumatic manifold can be interfaced with the microfluidic chip on any surface of the chip. The pneumatic manifold can be on the same or different side of the microfluidic chip as the cartridge. As shown in FIG. 1, a pneumatic manifold (113) can be placed on a surface of the microfluidic chip opposite to the cartridge. As well, the pneumatic manifold can be designed such that it only occupies a portion of the surface of microfluidic chip. The positioning, design, and/or shape of the pneumatic manifold can allow access of other components to the microfluidic chip. The pneumatic manifold can have a cut-out or annular space that allows other components to be positioned adjacent or proximal to the microfluidic chip. This can allow, for example, a magnetic component (109) to be placed in proximity of a chamber within the microfluidic chip.

[0096] A pneumatic manifold, or any other component described herein, can be constructed of any material known to those skilled in the art. For example, the cartridge can be constructed of a plastic, glass, or metal. Metals can include aluminum, copper, gold, stainless steel, iron, bronze, or any alloy thereof. The materials can be highly conductive materials. For example, a material can have a high thermal, electrical, or optical conductance. A plastic material includes any plastic known to those skilled in the art, such as polypropylene, polystyrene, polyethylene, polyethylene terephthalate, polyester, polyamide, poly(vinylchloride), polycarbonate, polyurethane, polyvinylidene chloride, cyclic olefin copolymer, or any combination thereof. The pneumatic manifold can be formed using any technique known to those skilled in the art, such as soft-lithography, conventional lithography, milling, molding, drilling, etching, or any combination thereof.

[0097] FIG. 13 shows the overall organization of a system. A microfluidic chip can be sandwiched between polycarbonate (PC) Pneumatic and Fluidic Manifolds. In this system, pipette tips (not shown) can be inserted into the top of the fluidic manifold and can serve both as fluid input/output ports, and as incubation reservoirs. The aluminum TEC-Tip Manifold can surround the four pipette tips that serve as incubation reservoirs (for Out1 and Out2) and controls their temperature with attached Peltier thermoelectric coolers (TECs). Note that although FIG. 13 shows two TEC Stacks, four TEC Stacks can be used. The other two TEC Stacks can be attached in similar positions, on the opposite face of the Tip Manifold. FIG. 21 shows a photograph of the system without pipette tips or TEC-Tip Manifold. The system can be assembled with bolts and thumb screws that serve to align the two manifolds and compress o-rings carried on the Pneumatic Manifold.

[0098] A Pneumatic Manifold can make a connection to pneumatic wells along the chip bottom surface. Gas-tight connections can be established with o-rings, glued to recesses on the top surface of the manifold. Each pneumatic chip well can then be connected, via through-holes in the manifold with glued-in metal canula (not shown), to a pneumatic line originating at a two-position solenoid valve. As described below, computer-controlled solenoid valves may select either vacuum or positive pressure for each pneumatic well. The Pneumatic Manifold can also carry two magnets interfacing with chip BPumps. FIG. 22 shows a Pneumatic Manifold with cutouts for (Delrin) Magnet Cradles carrying angled small